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Effect of oestradiol benzoate on oestrus intensity and pregnancy rate in CIDR treated anoestrus nulliparous and multiparous buffalo

Muhammad Rizwan Yousuf[a], J.P.N. Martins[b], Ali Husnain[a], Umair Riaz[a], Hasan Riaz[d], Abdul Sattar[a], Khalid Javed[c], Nasim Ahmad[a,*]

[a] Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, 54000 Pakistan
[b] Department of Animal Sciences, Michigan State University, East Lansing, 48824, USA
[c] Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore, 54000, Pakistan
[d] Department of Bio Sciences, COMSATS Institute of Information Technology, Sahiwal, 57000, Pakistan

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ABSTRACT

The objective of the present study was to examine if administration of oestradiol benzoate (OEB) after removal of control internal drug releasing device (CIDR) could enhance oestrous intensity and pregnancy rate in anovular nulliparous and multiparous Nili-Ravi buffalo. For this, a total of 298 nulliparous (n = 91) and multiparous (n = 207) buffaloes received a CIDR on Day 0, and were administered $PGF_{2\alpha}$ on Day 6 followed by removal of CIDR on Day 7. At Day 8, OEB was administered in approximately half of nulliparous (n = 45/91) and multiparous (n = 100/207) buffalo. All animals were fixed time inseminated 48 and 60 h after CIDR removal, respectively. The results showed that administration of OEB but not the parity, improved oestrous intensity (3.15 ± 0.05 vs 2.99 ± 0.05; P = 0.0026) compared to those not received OEB, respectively. However, OEB did not affect (46.2 vs 44.1; P = 0.8) pregnancy per AI (P/AI). In addition, P/AI was greater (50.7 vs 39.6; P = 0.036) in multiparous compared to nulliparous buffalo, respectively. The oestrous intensity (P = 0.025) and response to OEB (P = 0.0002) was greater in buffalo having a greater body condition (>3.0). Though, non significant, timing of ovulation was more synchronous (62.9 ± 1.8 vs 72.4 ± 3.6 h; P > 0.05) and ovulation rate was greater (91% vs 64%; P > 0.05) in buffalo after OEB administration. It is concluded that administration of OEB in conjunction with the CIDR improves oestrous intensity without affecting P/AI in nulliparous and multiparous anovular buffalo.

1. Introduction

Buffalo have an integral role in strengthening the agricultural economy, and improving food security to farmers in many Asian countries. They are excellent converters of low quality roughages and well adapted to harsh weather (Gordon, 1996). Despite these qualities, some of the main reproductive problems include delayed puberty in heifers, poor oestrous expression, anoestrus, long calving intervals and seasonality in breeding (Singh et al., 2000). Accumulated evidence suggests that these reproductive problems are associated with smaller ovaries and its structures, which release less estradiol and progesterone concentrations, eventually limiting breeding values of buffalo by late puberty, ovarian hypo-function and pathologies (Warriach et al., 2008; Purohit, 2014). Therefore, in an attempt to overcome such problems hormonal treatments have been used for optimization of ovarian functions.

* Corresponding author. Tel.: +92 42 99213659; fax: +92 42 99211461.
E-mail address: drnasim@yahoo.com (N. Ahmad).

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Most of the oestrous synchronization protocols in buffalo are empirically based on those developed for cattle by inducing premature luteolysis using prostaglandins (Kumaratillake et al., 1977; Pathiraja et al., 1979) or prolonging the luteal phase using progestagens (Martinez et al., 1997; Martinez et al., 2000, 2002) which ultimately resulted in variable fertility. Among many of these oestrous synchronization protocols the use of CIDR device for reproductive management in buffalo is most prevalent, as few previous studies achieved comparable pregnancy rates in anoestrous buffalo during breeding or non-breeding seasons (Murugavel et al., 2009; Ahmad et al., 2010; Naseer et al., 2011; Carvalho et al., 2013; Barlie et al., 2015). The use of the CIDR has resulted in a resumption of oestrous cycles in anovular cows (Rhodes et al., 2002) and Holstein heifers (Yusuf et al., 2010). However, the effectiveness of these oestrous synchronization protocols appears to be limited in buffalo because farmers have to rely on visual methods of oestrous detection, which is not efficient in most of the buffalo herds or commercial farms. To overcome this, administration of OEB after removal of CIDR allows for a timed artificial insemination (TAI) program with optimal pregnancy rates (~60%) in a limited number of buffalo (Naseer et al., 2011). This approach induced acceptable ovulation rates (~80%) with greater synchrony of behavioral oestrus (Sales et al., 2012). Perhaps the addition of OEB in CIDR-treated buffalo optimizes the secretions of oestradiol which are at suboptimal concentrations due to either size of the mature follicle or insufficiency of neural mechanism controlling oestrous expression and hormone synthesis. It is likely, that anoestrous buffalo would have a similar hormonal status. Therefore, in the present study, oestrous intensity, ovulation synchrony and pregnancy rate were assessed following OEB administration in CIDR-treated nulliparous and multiparous buffalo and associations with body condition score (BCS) were assessed.

2. Materials and methods

2.1. Animals

The experiment was conducted at commercial dairy farms in the breeding season (September to December) in years 2011–2013 in the suburb of District Lahore, Pakistan. Anoestrous, adult (multiparous) Nili-Ravi buffalo (n = 207) 4 to 7 (5.8 ± 1.3) years of age and with body weights (BW) of 450 to 550 kg, and a BCS of 3.25 ± 0.38 were managed and fed under optimal conditions. Similarly, buffalo heifers (n = 91, nulliparous) 3.1 ± 1.4 years of age with a BW of more than 350 kg (BCS, 3.25 ± 0.44), and with no history of any oestrous signs were selected and fed under optimal conditions. Each buffalo was scored for body condition as described elsewhere (Alapati et al., 2010). For further confirmation of the anoestrus ovarian ultrasonography (Honda HS-1500V with 10 MHz probe) was conducted twice within a 14-day interval to ascertain whether there was a functional CL present or not. At the start of experiment, both uterine horns were assessed using ultrasonography for any unexpected pregnancies or any uterine abnormalities.

2.2. Oestrous synchronization protocol

Briefly, CIDR devices (1.38 g progesterone; Eazi-breed®, New Zealand) were placed in the anterior vagina of all selected buffalo and this date was designated as Day 0. On Day 6, all animals received a single dose of PGF2α (0.150 mg; Dalmazine, cloprosteonol, Fatro®, Italy; 2 ml; i.m.) and after 24 h (Day 7) the CIDR was removed. To compare efficacy of OEB, approximately half of multiparous (100 of 207) and nulliparous (45 of 91) buffalo were randomly injected with 4 ml of OEB, 24 h after CIDR removal (0.4 mg; Oestradiol benzoate; Sigma®, St. Louis, MO, USA). Ovarian ultrasonography on Day 7 (CIDR removal day), Day 8, Day 9 (morning and evening) and Day 10 was conducted to measure the diameter of the largest follicle and to confirm ovulation.

2.3. Measurement of oestrous intensity

All the animals were examined for the oestrous intensity with the scale of 1 to 5 in ascending order (1 = poor and 5 = excellent), after slight modifications as reported earlier by Van Eerdenberg et al. (1996) (Table 1).

2.4. Trans-rectal ultrasonography, AI and pregnancy diagnosis

The follicular dynamics was monitored using the Honda HS-1500 V ultrasonography machine having a tran-srectal probe (10 MHz). The follicular diameter was measured twice a day from the day of CIDR removal until disappearance of the largest follicle (>9 mm, apparent ovulation). Each animal was artificially inseminated twice using frozen–thawed semen from a single bull (Semen Production Unit, Qadirabad, Punjab, Pakistan) of known fertility at approximately 48 and 60 h after removal of the CIDR. On Day 40 after insemination all the buffalo were screened for pregnancy by using trans-rectal ultrasonography. The pregnancy was based on the presence of fetal heart beat and amniotic fluid.

Table 1
Oestrous intensity score based on the animal's characteristics.

Oestrous intensity score	Grade	Characteristics
1	Poor	No uterine tone with no behavioral signs
2	Satisfactory	Mild uterine tone, slight mucus discharge, some restlessness
3	Good	Intermediate uterine tone, mucus discharge, restlessness, nervousness
4	Very good	Good tone, stand to be mounted, vulvar swelling, thick mucus discharge, restlessness
5	Excellent	High tone, stand to be mounted, thick mucus discharge, restless

Table 2
Effect of parity and oestradiol benzoate on oestrous intensity and pregnancy per AI in nulliparous and multiparous buffalo.[a]

	Nulliparous		Multiparous		P^b		
	No EB	EB	No EB	EB	A	E	A × E
Oestrous Intensity	3.0 ± 0.04	3.10 ± 0.06	2.97 ± 0.05	3.21 ± 0.05	0.45	0.0026	0.21
Pregnancy/AI (%)	41.3 (19/46)	37.8 (17/45)	47.2 (50/106)	54.5 (55/101)	0.036	0.80	0.35

[a] All buffalo were subjected to CIDR based estrus synchronization protocol with a single dose of PGF2α 24 h before CIDR removal. Some buffalo were injected with 4 ml of oestradiol benzoate (OEB) 24 h after CIDR removal and remaining buffalo were grouped as “No OEB”.
[b] A represents effect between nulliparous compared with multiparous; E, effect between OEB and No OEB; A × E, interaction between parity and treatment.

Table 3
Effects of BCS and oestradiol benzoate on oestrous intensity and pregnancy per AI in nulliparous and multiparous buffalo.[a]

	BCS < 3		BCS > 3		P^b		
	No OEB	OEB	No OEB	OEB	B	E	B x E
Estrus Intensity	2.94 ± 0.05	3.05 ± 0.04	3.09 ± 0.06	3.46 ± 0.09	0.025	0.0002	0.6306
Pregnancy/AI (%)	48.7 (40/78)	48.6 (33/68)	41.8 (31/74)	57.8 (45/78)	0.4940	0.5190	0.531

*(BCS = 1 for thin and 5 for fat).
[a] All buffalo were subjected to CIDR based oestrous synchronization protocol with a single dose of PGF2α 24 h before CIDR removal. Some buffalo were injected with 4 ml of oestradiol benzoate (OEB) 24 h after CIDR removal and remaining buffalo were grouped as “No OEB”.
[b] represents body condition score (BCS < 3 compared with BCS > 3); E, effect between OEB and No OEB; B × E interaction between BCS and treatment.

2.5. Statistical analysis

The data were analyzed with the statistical software (SAS Inst. Inc. Carey, NC). The data regarding oestrous intensity and pregnancy/AI were analyzed by using logistic regression (procedure Proc logistic SAS Inst. Inc. Carey, NC) with the model consisting of body condition score (BCS), parity and treatment (OEB or No OEB). A stepwise backward elimination was used based on the Wald statistics criterion when $P > 0.10$. The significance level for each variable was set at $P < 0.05$.

3. Results

Data for the effect of parity and OEB on oestrous intensity and pregnancy per AI (P/AI) of buffalo in which time of oestrus was synchronized with the CIDR-based protocol are presented in Table 2. Overall, addition of OEB improved ($P = 0.0026$) oestrous intensity compared with buffalo not treated with OEB. Interestingly, when the OEB treatment was not considered P/AI was greater in multiparous buffalo compared with nulliparous buffalo (50.7 compared with 39.6; $P = 0.036$), however, OEB appeared to have no effect on P/AI (46.1 compared with 44.2; $P = 0.8$). No significant interaction was observed between parity and OEB in relation to oestrous intensity score or P/AI in these buffalo.

The data for effect of BCS and OEB on oestrous intensity and P/AI are presented in Table 3. The results showed that when administration of OEB was not considered oestrus intensity was greater ($P = 0.025$) in buffalo (BCS > 3) with greater body energy reserves. Similarly, OEB treatment improved oestrous intensity score compared to animals that did not receive OEB ($P = 0.0002$). However, no interaction was observed between BCS and OEB in improving oestrous intensity score and P/AI.

In addition, treatment of OEB on changes in follicle size, timing of ovulation and ovulation rate were also studied. The results showed that ovulatory diameter did not vary between groups and ranged 14.2–18.8 mm in OEB and control groups. Similarly, OEB had no significant effect ($P > 0.05$) on ovulation rate, or timing of ovulation (Table 4).

4. Discussion

The rationale for conducting the present experiment was based on a preliminary study conducted by Naseer et al., 2011 on a relatively smaller buffalo herd ($n = 37$) which reported that incorporation of OEB would potentially facilitate fixed time artificial insemination (FTAI) with substantial increase in pregnancy rate in anoestrous buffalo. In the present study, a relatively large number of nulliparous and multiparous buffalo were used in the study and data indicate that administration of OEB after CIDR removal enhances oestrous intensity, and time of ovulation synchrony which facilitated FTAI without affecting pregnancy rate in anoestrous buffalo. These findings are important for the reproductive management of buffalo herds, especially in reducing age at first calving in buffalo heifers and decreasing the post-partum calving interval in case of adult buffalo.

Interestingly, in the present study all animals assigned to the CIDR protocol showed signs of oestrus. These results are consistent with those of previous studies where oestrous response after CIDR removal was 97% in anoestrous Nili-Ravi buffalo, and was 100% in anoestrous

Table 4
Effect of oestradiol benzoate (OEB) on follicle size, timing of ovulation and ovulation rate in buffalo in which time of oestrus was synchronized with a CIDR based protocol.

Variables	No OEB	OEB
Size of follicle at OEB inj. time (mm)	11.9 ± 0.9	10.7 ± 0.5
Ovulatory follicle size (mm)	17.4 ± 0.5	16.3 ± 0.4
Timing of ovulation-hours (range)	72.9 ± 3.6	62.4 ± 1.8
Ovulation rate	64% (7/11)	91% (10/11)

Egyptian buffalo using the CIDR with administration of GnRH (Zaabel et al., 2009; Naseer et al., 2011). Furthermore, there was greater oestrous synchrony and greater ovulation rates achieved both in nulliparous and multiparous buffalo after OEB treatment, which could be very conducive to implementation of a FTAI program, thus, eliminating time and labor required for detection of oestrus. These results have wider applications; particularly to those buffalo in which FTAI is not envisaged because the oestrous signs will be improved allowing AI at oestrus which most likely will increase fertility. Findings for investigations in the present study are consistent with those of Azawi et al. (2012) and Yotov et al. (2012) who described that buffalo submitted to PRID or CIDR treatment protocols had greater secondary oestrous activity than those treated with the "ovsync" program (Zaabel et al., 2009).

Results of the present study indicate that there was a greater influence of OEB treatment on oestrous intensity in adult buffalo than buffalo heifers. The most likely reason for lesser oestrous expression in nulliparous buffalo could be the dosage of estradiol, which is unable to sensitize hypothalamus for induction of behavioral signs of oestrus or maybe there was no synchrony in follicular wave emergence resulting in smaller diameters of follicles from which ovulation occurred. Another explanation could be the small number of nulliparous buffalo used in the present experiment. Despite the fact that oestrous expression was limited in buffalo heifers, the CIDR treatment induced oestrus in all of these animals hence the CIDR treatment can be successfully used with insemination at a detected oestrus or in fixed time artificial insemination programs to improve reproductive efficiency in buffalo heifers.

In the present study, treatment with OEB did not affect pregnancy rate in these buffalo but the overall pregnancy rate was consistent with results from a previously reported study in Nili-Ravi buffalo (50% compared with 59%) (Naseer et al., 2011). Interestingly, P/AI was considerably less in nulliparous than multiparous buffalo. The most probable reason of this could be inappropriate body weight of buffalo heifers and lesser ovulation rate due to insufficiency of LH. A previous study reported that a majority of buffalo heifers initiate oestrous cycles with optimum pregnancy rate in subsequent oestrous cycles (Ahmad et al., 2010). Results of the present study are encouraging due to the fact that use of the CIDR and OEB enhances oestrous intensity without affecting the fertility of postpartum anoestrous buffalo.

Body condition score (BCS) of animals could affect oestrous expression and hormonal imbalance due to improper growth of ovarian follicles caused by a negative energy balance. Results of the present study indicate that OEB was more effective in buffalo with BCS of >3 for inducing oestrous expression. These findings indicate that use of the CIDR and OEB protocol should occur when body condition scores of buffalo are considered.

In conclusion, a CIDR device can be successfully used to induce oestrus in anoestrous buffalo. Furthermore, incorporation of OEB after CIDR removal appeared to be an effective tool to enhance oestrous expression with optimum pregnancy rate by fixed time AI in adult buffalo. In addition, inclusion of the CIDR and OEB in treatment programs should be based on body condition status of buffalo.

Conflict of interest

There is no conflict of interest among authors regarding this manuscript.

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References

Ahmad, N., Ahmad, E., Naseer, Z., 2010. Use of CIDR for the regulation of fertility in Anoestrous buffaloes. In: Proceedings 9th World Buffalo Congress, Buenos Aires, pp. 997–1000.

Alapati, A., Kapa, S.R., Jeepalyam, S., Rangappa, S.M., Yemireddy, K.R., 2010. Development of the body condition score system in Murrah buffaloes: validation through ultrasonic assessment of body fat reserves. J. Vet. Sci. 11 (1), 1–8.

Azawi, O.I., Ali, M.D., Oday, S.A., Al-Hadad, A., Mouayad, S., Salman, A., Hadad, A., 2012. Treatment of Anoestrous in Iraqi Buffaloes using Ovsynch alone or in Combination with CIDR. J. Adv. Vet. Res. 2, 68–72.

Barlie, V.L., Terzano, G.M., Pacelli, C., Todini, L., Malfitti, A., Barbato, O., 2015. LH peak and ovulation following two different oestrous synchornization treatments in buffalo cows in the daylight lengtheing period. Theriogenology, http://dx.doi.org/10.1016/j.theriogenology.2015.03.019.

Carvalho, N.A.T., Soares, J.G., Porto, Filho, R.M., Gimenes, L.U., Souza, D.C., Nichi, M., Sales, J.S., Baruselli, P.S., 2013. Equine Chorionic gonadotrophin impores the efficacy of a timed artificial insemination protocol in buffalo during the nonbreeding season. Theriogenology 79, 423–428.

Gordon, I., 1996. Controlled Reproduction in Cattle and Buffaloes. CAB International, Willingford, UK.

Kumaratillake, W.L., Pathiraja, N., Perera, B.M., Tilakeratne, N., 1977. Synchronisation of oestrus in buffaloes (*Bubalus bubalis*) using prostaglandin F2alpha. Res. Vet. Sci. 22 (3), 380–381.

Martinez, F.M., Kastelic, J.P., Adam, G.P., Janzen, E., McCartney, D.H., Mapletoft, R.J., 1997. Oestrus synchronization and Pregnancy rate in Beef cattle given CIDR-B, prostaglandin and Estradiol or GnRH. Can. Vet. J. 41, 786–790.

Martinez, M.F., Kastelic, J.P., Adams, G.P., Janzen, E., McCartney, D.H., Mapletoft, R.J., 2000. Oestrus synchronization and pregnancy rates in beef cattle given CIDR-B, prostaglandin and estradiol, or GnRH. Can. Vet. J. 41 (10), 786–790.

Martinez, M.F., Kastelic, J.P., Adams, G.P., Mapletoft, R.J., 2002. The use of a progesterone-releasing device (CIDR-B) or melengestrol acetate with GnRH, LH, or estradiol benzoate for fixed-time AI in beef heifers. J. Anim. Sci. 80 (7), 1746–1751.

Murugavel, K., Antoine, D., Raju, M.S., Lopez-Gatius, F., 2009. The effect of addition of equine chorionic gonadotropin to a progesterone-based oestrous synchronization protocol in buffaloes (*Bubalus bubalis*) under tropical conditions. Theriogenology 71, 1120–1126.

Naseer, Z., Ahmad, E., Singh, J., Ahmad, N., 2011. Fertility following CIDR based synchronization regimens in anoestrous Nili-Ravi buffaloes. Reprod. Domest. Anim. 46 (5), 814–817.

Pathiraja, N., Abeyratne, A.S., Perera, B.M., Buvanendran, V., 1979. Fertility in buffaloes after oestrus synchronisation with cloprostenol and fixed time insemination. Vet. Rec. 104 (13), 279–281.

Purohit, G.N., 2014. Ovarian and oviductal pathologies in the buffalo: occurance, diagnostic and therapeutic approaches. Asian Pac. J. Reprod. 3 (2), 156–168.

Rhodes, F.M., Burke, C.R., Clark, B.A., Day, M.L., Macmillan, K.L., 2002. Effect of treatment with progesterone and oestradiol benzoate on ovarian follicular turnover in postpartum an oestrous cows and cows which have resumed oestrous cycles. Anim. Reprod. Sci. 69 (3–4), 139–150.

Sales, J.N., Carvalho, J.B., Crepaldi, G.A., Cipriano, R.S., Jacomini, J.O., Maio, J.R., Souza, J.C., Nogueira, G.P., Baruselli, P.S., 2012. Effects of two estradiol esters (benzoate and cypionate) on the induction of synchronized ovulations in Bos indicus cows submitted to a timed artificial insemination protocol. Theriogenology 78 (3), 510–516.

Singh, J., Nanda, A.S., Adams, G.P., 2000. The reproductive pattern and efficiency of female buffaloes. Anim. Reprod. Sci. 60–61, 593–604.

Van Eerdenberg, F.J.C.M., Loeffler, H.S.H., Van vilet, J.H., 1996. Detection of oestrus in daily cows, a new approach to an old problem. Van. Q. 18, 52–54.

Warriach, H.M., Channa, A.A., Ahmad, N., 2008. Effect of oestrus synchronization methods on oestrus behaviour, timing of ovulation and Pregnancy rate during breeding and low breeding season in Nili-Ravi buffaloes. Anim. Reprod. Sci. 107, 62–67.

Yotov, S., Atanasov, A., Ilieva, Y., 2012. Therapy of ovarian inactivity in postpartum Bulgarian Murrah buffaloes by PRID and Ovsynch oestrus synchronization protocols. Asian Pac. J. Reprod. 1, 293–299.

Yusuf, M., Nakao, T., Yoshida, C., Long, S.T., Fujita, S., Inayoshi, Y., Furuya, T., 2010. Comparison in effect of Heatsynch with heat detection aids and CIDR-Heatsynch in dairy heifers. Reprod. Domest. Anim. 5 (3), 500–504.

Zaabel, S.M., Hegab, A.O., Montasser, A.E., El-Sheikh, H., 2009. Reproductive performance of anoestrous buffaloes treated with CIDR. Anim. Reprod. 6 (3), 460–464.